The Stromal and Immune Landscape of Colorectal Cancer Progression during Anti-EGFR Therapy

The stromal and immune landscape of colorectal cancer progression during anti-EGFR therapy

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In this issue of *Cancer Cell*, Woolston et al. show that colorectal cancers that become refractory to initially effective anti-EGFR therapy, besides harboring resistance-conferring mutations, also contain abundant stromal and immune cells. This phenotypic reconfiguration has functional relevance and puts forward therapeutic opportunities for patients who relapse on EGFR-targeting treatment.

The EGFR antibodies cetuximab and panitumumab are used in patients with *KRAS* or *NRAS* wild-type metastatic colorectal cancer (mCRC) either in combination with standard chemotherapy, for first-line treatment, or as single agents when tumors become resistant to prior cytotoxic regimens. However, only 20% of individuals experience tumor regressions, and only an additional 30% have some extent of clinical benefit in terms of disease stabilization (Douillard et al., 2013). This relatively low response rate is compounded by the dismal reality that subjects who initially respond typically become refractory to treatment in a period of months. In this issue of *Cancer Cell*, Woolston et al. (2019) offer a comprehensive picture of the identifying traits of primary and acquired resistance to cetuximab in a clinical cohort of 35 mCRC patients (Figure 1). Different from previous studies, mostly conducted in a retrospective manner and focused on a small number of candidate biomarkers, here the authors embarked on a prospective trial whereby biopsies collected before initiation of single-agent cetuximab and at the time of disease progression were subjected to whole exome and RNA sequencing analyses and immunophenotyping.

Lack of response to antibody treatment *ab initio* has been partly ascribed to the occurrence of mutations or amplifications in genes encoding other tyrosine kinase receptors or RAS downstream effectors, which, similar to mutationally activated RAS, trigger compensatory pathways sustaining EGFR-independent tumor growth (Bertotti et al., 2015). Since these genetic abnormalities occur individually at very low frequency, their catalog has not yet saturated the space of mCRC tumors with primary resistance to EGFR antibodies. Indeed, when the authors stratified global genomic data onto response annotation, they pinpointed previously unrecognized alterations, including biallelic inactivation of *NF1* (a GTPase-activating protein that antagonizes RAS function) and *KRAS* and *BRAF* mutations endowed with attenuated enzymatic and transforming activity. Interestingly, hypomorphic *BRAF* and *KRAS* mutations co-existed in the same tumor or, when present singly, were associated with polysomy of the corresponding chromosome, suggesting that their individual contribution to cetuximab resistance is suboptimal and requires either a cooperative or a dosage effect for complete manifestation.

Secondary resistance is often propelled by the clonal expansion of the same alterations responsible for primary resistance, with a preponderance of RAS pathway mutations. Such alterations may arise *de novo* on a stochastic basis, as a consequence of tumor genetic instability, or may pre-exist as minor subclones in the original tumor population because of genetic

heterogeneity and become positively selected under drug pressure (Khan et al., 2018). Genetic instability and heterogeneity explain why acquired resistance mutations are usually polyclonal and can be more accurately grasped by analysis of circulating tumor DNA (ctDNA) – which incorporates DNA fragments shed by the whole tumor – than by examination of solid biopsies – which, by definition, are subspatial snapshots of the entire lesion (Khan et al., 2018). Woolston et al. (2019) report a number of genetic alterations of acquired resistance, including already characterized mutations in components of the RAS pathway (Khan et al., 2018) and a hitherto unidentified amplification of *FGF10* (encoding a ligand of the FGFR2 tyrosine kinase receptor). These genetic aberrations were detected in only a limited number of post-treatment biopsies but were mostly captured in ctDNA samples, further attesting to the pervasiveness of tissue sampling bias. Of note, ctDNA resistance mutations were calculated to occur in a minority of cells, in keeping with previous reports demonstrating the presence of recurrent but subclonal RAS pathway mutations in the blood of cetuximab-refractory mCRC patients (Bettegowda et al., 2014; Khan et al., 2018). Altogether, these findings suggest that tumor relapse is engendered by polyclonal mutuality, with an ecosystem of different subclones contributing to therapeutic resistance. However, we cannot exclude that when subclonal alterations are present at a very low allele frequency the impact on resistance may remain sub-threshold, and other (non-genetic) determinants could subsidize DNA mutations to reduce responsiveness to EGFR inhibition.

Woolston *et al*. (2019) strongly embrace the assumption that progression on cetuximab can be also fostered by non-mutational mechanisms and extend their investigation by delineating the transcriptomic profiles of matched sensitive and post-therapy resistant tumors. First, they confirm that a subgroup of tumors with gene expression traits reminiscent of those portrayed by the transient-amplifying precursors of the normal intestine (assigned to the so-called CMS2 consensus transcriptional subtype) were enriched for cetuximab-responsive cases. Then, the authors show that the majority of tumors with acquired resistance to cetuximab (including some harboring subclonal mutations) underwent a gene expression transition towards a stroma-rich (CMS4) phenotype featuring high content of carcinoma-associated fibroblasts (CAFs) and increased expression of CAF-derived growth factors such as TGFβ, HGF, and FGF family ligands. Consistent with the observed association between stromal abundance and drug resistance, the CAF secretome was found to exert a protective activity against cetuximab. These results highlight a key role for transcriptionally regulated growth factors in conveying survival cues that safeguard CRC tumors from the effects of EGFR blockade, in agreement with previous findings (Zanella et al., 2015).

Intriguingly, Woolston *et al*. (2009) describe a more copious representation of cytotoxic T lymphocytes and dendritic cells, increased expression of a T cell-associated inflammatory signature, and upregulation of immune checkpoints in CMS4-like, TGFβ-high resistant tumors. This result corroborates a retrospective study documenting heightened infiltration of cytotoxic, effector

memory, and regulatory T cells in CRC tumors treated with cetuximab and chemotherapy (Van den Eynde et al., 2018). However, the coexistence of elevated TGFβ activity and immune inflammation is unexpected, as TGFβ is known to impair T cell function and promote T cell physical exclusion (Tauriello et al., 2018). How can cetuximab-resistant tumors concomitantly display high levels of immune suppressive TGFβ and an active immune microenvironment? One possibility is that increased immune infiltration precedes the CMS2/CMS4 transition. This would be coherent with the notion that cetuximab triggers IgG1 antibody-mediated immunogenic cell death (Pozzi et al., 2016) and with the observation that EGFR pathway activity in lung cancer prompts immune escape, which is counteracted by EGFR inhibition (Akbay et al., 2013). If this is the case, immune stimulation would be a direct consequence of productive cetuximab treatment rather than a hallmark of cetuximab resistance, and it would be interesting to see whether cetuximab-sensitive tumors at maximal response to EGFR blockade have already undergone the inflammatory shift shown by resistant tumors. One could even push this reasoning to the extreme: strengthened TGFβ activity might be a delayed adaptive mechanism to contrast cetuximab-induced immune cell deployment. In this scenario, immunotherapy is expected to synergize with cetuximab in the early phases of response rather than after the emergence of resistance.

The application of genomic technologies has enabled the identification of clinically actionable DNA alterations in RAS wild-type mCRC tumors that fail or cease to respond to EGFR antibodies, including those illustrated in this study. By providing fresh evidence that acquired resistance to cetuximab also entails a stromagenic and immune-inflamed phenotype, results from Woolston *et al*. have important ramifications for the biological understanding of CRC evolution under EGFR blockade and introduce potential opportunities for targeting a novel repertoire of non-mutational vulnerabilities.

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Declaration of Interests:

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Figure legend

Figure 1. The landscape of cetuximab resistance in mCRC, as reported by Woolston et al. (2019). New genetic alterations are found to be associated with, and causally responsible for, treatment failure in tumors that are or become insensitive to cetuximab therapy. Furthermore, tumors with acquired resistance present an abundance of stromal growth factors, which protect cancer cells from the antiproliferative effects of EGFR inhibition. Finally, tumors from patients who progress on cetuximab are more infiltrated by immune cells and have higher expression levels of immune checkpoints than tumors from cetuximab-naïve patients.

References

1. Akbay, E.A., Koyama, S., Carretero, J., Altabef, A., Tchaicha, J.H., Christensen, C.L., Mikse, O.R., Cherniack, A.D., Beauchamp, E.M., Pugh, T.J., et al. (2013). Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. Cancer Discov. *3*, 1355-1363.
2. Bertotti, A., Papp, E., Jones, S., Adleff, V., Anagnostou, V., Lupo, B., Sausen, M., Phallen, J., Hruban, C.A., Tokheim, C., et al. (2015). The genomic landscape of response to EGFR blockade in colorectal cancer. Nature *526*, 263-267.

3. Bettegowda, C., Sausen, M., Leary, R. J., Kinde, I., Wang, Y., Agrawal, N., Bartlett, B. R., Wang, H., Luber, B., Alani, R. M., et al. (2014). Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci. Transl. Med. *6*, 224ra224.
4. Douillard, J.Y., Oliner, K.S., Siena, S., Tabernero, J., Burkes, R., Barugel, M., Humblet, Y., Bodoky, G., Cunningham, D., Jassem, J., et al. (2013). Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N. Engl. J. Med. *369*, 1023-1034.
5. Khan, K.H., Cunningham, D., Werner, B., Vlachogiannis, G., Spiteri, I., Heide, T., Mateos, J.F., Vatsiou, A., Lampis, A., Damavandi, M.D., et al. (2018). Longitudinal liquid biopsy and mathematical modeling of clonal evolution forecast time to treatment failure in the PROSPECT-C phase II colorectal cancer clinical trial. Cancer Discov. *8*, 1270-1285.
6. Pozzi, C., Cuomo, A., Spadoni, I., Magni, E., Silvola, A., Conte, A., Sigismund, S., Ravenda, P.S., Bonaldi, T., Zampino, M.G., et al. (2016). The EGFR-specific antibody cetuximab combined with chemotherapy triggers immunogenic cell death. Nat Med. *22*, 624-631.
7. Tauriello, D.V.F., Palomo-Ponce, S., Stork, D., Berenguer-Llergo, A., Badia-Ramentol, J., Iglesias, M., Sevillano, M., Ibiza, S., Cañellas, A., Hernando-Momblona, X., et al. (2018). TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. Nature *554*, 538-543.
8. Van den Eynde, M., Mlecnik, B., Bindea, G,, Fredriksen, T., Church, S.E., Lafontaine, L., Haicheur, N., Marliot, F., Angelova, M., Vasaturo, A., et al. (2018). The link between the multiverse of immune microenvironments in metastases and the survival of colorectal cancer patients. Cancer Cell *34*, 1012-1026.
9. Woolston, A., Khan, K., Spain, G., Barber, L.J., Griffiths, B., Gonzalez-Exposito, R., Hornsteiner, L., Punta, M., Patil, Y., Newey, A., et al. (2019). Genomic and transcriptomic determinants of therapy resistance and immune landscape evolution during anti-EGFR treatment in colorectal cancer. Cancer Cell *35*, XXX-XXX.
10. Zanella, E.R., Galimi, F., Sassi, F., Migliardi, G., Cottino, F., Leto, S.M., Lupo, B., Erriquez, J., Isella, C., Comoglio, P.M., et al. (2015). IGF2 is an actionable target that identifies a distinct subpopulation of colorectal cancer patients with marginal response to anti-EGFR therapies. Sci. Transl. Med. *7*, 272ra12.

PRIMARY RESISTANCE	ACQUIRED RESISTANCE		
DNA alterations	**DNA alterations**	**Transcriptomic changes**	**Immune contexture**
KRAS and *BRAF* hypomorphic mutations	*FGF10* amplification	CMS2>CMS4 transition	Increased infiltration of cytotoxic T cells and dendritic cells
		Higher expression of TGFβ, FGFs, HGF	Inflammatory signature
NF1 loss			Upregulation of immune checkpoints